family: vibrionaceae

Genus: Vibrio

General characteristics

- They are curved G -ve (comma shape) ,aerobic rods, motile with single polar flagellum, found in single or in cluster forming S shape, non spore former, on prolong cultivation Vibrio may become straight rods. Vibrio found in nature mostly in water, fishes and food.
- Culture: Vibrio produce convex ,smooth round colonies, opaque and granular in transmitted light,
- they are oxidase +ve,
- most Vibrio grow well at 37 °C on media containing mineral salt and amino acids (Aspargin, Arginine, Lysine) as a source of carbon and nitrogen.
- These organisms grow at alkaline pH(8.5-9.5) but they are rapidly killed by acid and heating at 55 0C for 15 min.,culture containing carbohydrates become sterile after few days. Vibrio cholera grows well on TCBS (Thiosulfate citrate bile sucrose) media.

General characteristics

- **These organisms grow at alkaline pH(8.5-9.5) but they are rapidly killed by acid and heating at 55 0C for 15 min.**
- **Vibrio cholera grows well on TCBS (Thiosulfate citrate bile sucrose) media.**

- Vibrio cholera (V.C.): 01,0139
- Non vibrio cholera (N.V.C.):02-0138
- V. parahaemolyticus (food-asscciated diarrhea disease)
- V. vulnificis(wound infection)
- V. alginolyticus (otitis externa,wound infection)

TABLE 35-1 Human infections caused by *Vibrio* species

Bacteria	Diseases
Vibrio cholerae	Diarrhea
Vibrio parahaemolyticus	Gastroenteritis and wound infections
Vibrio vulnificus	Gastroenteritis and wound infections
Vibrio hollisae	Gastroenteritis and wound infections
Vibrio mimicus	Gastroenteritis and wound infections
Vibrio metschnikovii	Bacteremia
Vibrio alginolyticus	Wound infections and otitis externa

Eltor	Classical
Hemolytic	Non Hemolytic
Polymixin B (resistant)	Polymixin B (sensitive)
VP. +ve	VP. - ve
Cause heme agglutination of sheep RBC	dose not

- **Laboratory Diagnostic tests:**
- **1-Gram stain.**
- **2- TCBS (Na-citrate ,Na-thiosulfate, bile salt,sucrose,bromothymolblue,pH:8)**
- **3- Peptone water Nacl 7%, 0%**
- **4- IMVIC**
- **5- Motility**
- **6- Nitrate reduction test.**
- **7-TSI test**
- **8- OF media.**
- **9- Mannitol fermentation.**
- **10 - Catalase and oxidase test.**

- **11 - String test : When an isolated colony (18-24 hour growth) of a suspected bacterium is emulsified in Sodium deoxycholate or Sodium taurocholate (commonly known as bile salt), it lyses the cell wall of the bacterium releasing the DNA . Take a clean grease free slide and put a drop of (0 .5% bile salt). Emulsify an isolated colony of the bacterium using an inoculating loop. Keep on rubbing the loop vigorously for 2-3 min. until it appears viscous. Gently, pull the loop upwards from the slide. Formation of a thread like mucoid string indicates a positive test.**
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Positive String Test

- 12- Cholera red test. Tryptophane + cone. H_2SO_4 ----+ nitrosoindole (red color +ve)

  {Reagent :Sulphuric acid (1-3) drops}

Media : peptone water(18-24)h.

- 13- Oxidase test (4,5 tetramethyl paraphenyl diamine dihydro chloride),The oxidase test is based on the bacterial production of an oxidase enzyme. Transfer 1 colony (not from blood agar) to filter paper by loop then add 1 drop of (4,5 tetramethyl paraphenyl diamine dihydro chloride) +ve dark violet,,,,,,,, - ve no change in color

Biochemical reactions

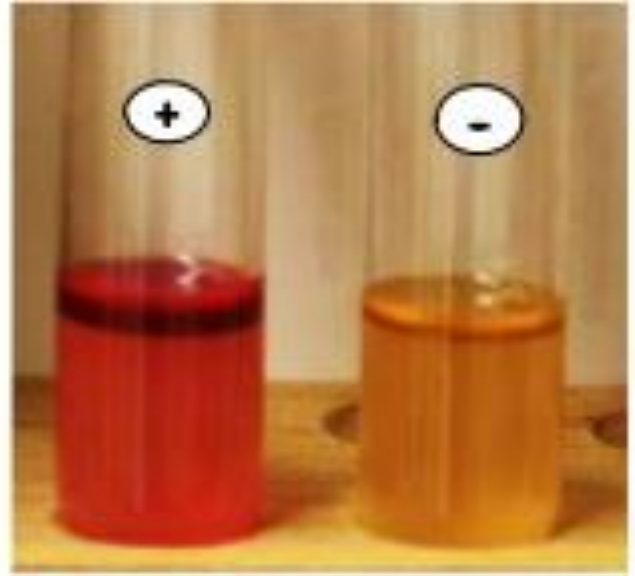
- Cholera red reaction
  - Ferments glucose, mannitol, maltose, mannose and sucrose
  - Produces indole which gives red color

Biochemical tests

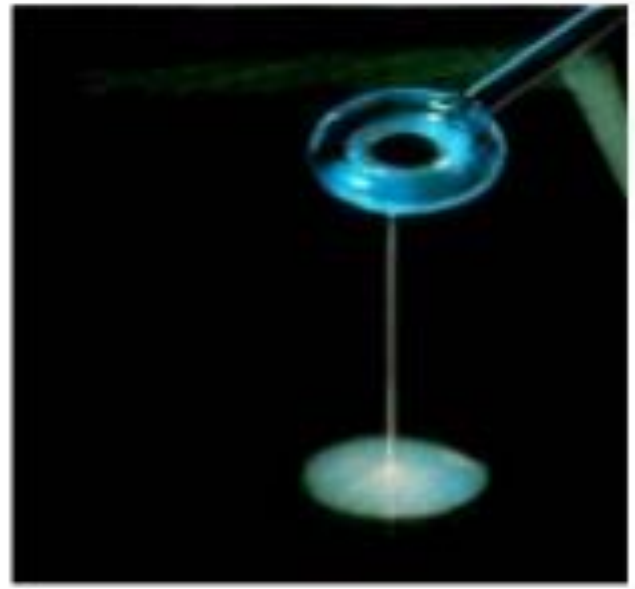
- Ferment glucose sucrose maltose but not Lactose (only acid no gas)
- Indole +ve
- Nitrate reduced | nitroso-indole

(CHOLERA RED REACTION)

- MR –ve; VP +ve in ElTor
- Urease –ve
- Oxidase +ve
- Catalase +ve
- Lysine and Ornithine decarboxylated
- Gelatin is liquefied
- String test +ve



Concentrated sulphuric acid



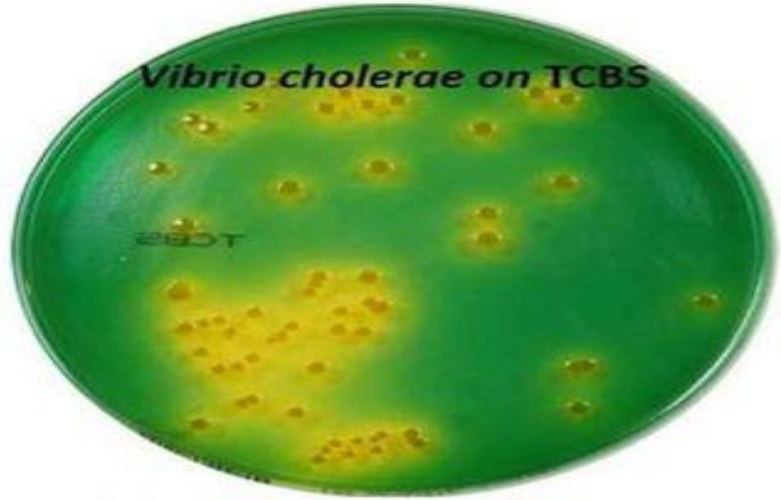
0.5% sodium deoxycholate

Stool culture

Use Cary Blair Transport media if available – Viable for many days at room temperature • Use TCBS media(Thiosulfate-citrate-bile salts-sucrose agar)

for culture it's selective media for vibrio cholera

Alkaline peptone water is ideal enrichment medium



Vibrio cholerae on TCBS Agar

Vibrio parahaemolyticus on TCBS Agar

Selective Medium - TCBS

- V.cholrae grows well on Thiosulphate citrate bile sucrose (TCBS) agar, on which it produces yellow colonies that are readily visible against the dark green background of the agar.

SELECTIVE MEDIA

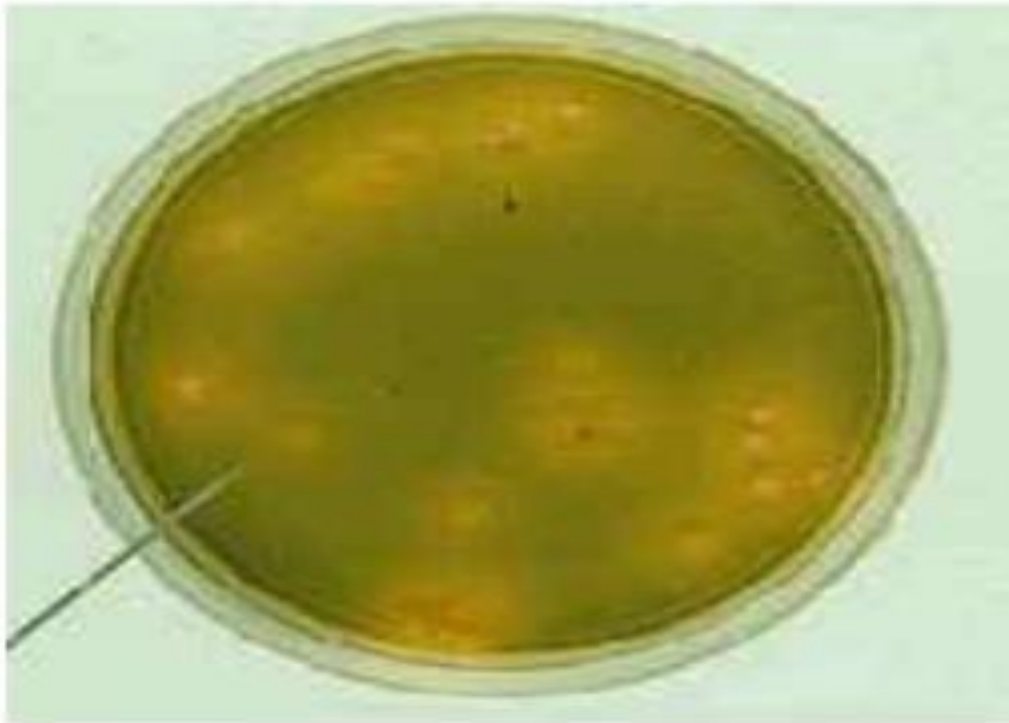
- It is a medium in which certain substances are present which inhibit all other bacteria except the desired bacteria.
- It encourages the growth of particular species from a mixed inoculum.

Example:- TCBS

- It is light green translucent medium kept in petridish
- It is selective medium for *Vibrio cholera*
- **Principle:-**

  Bile salt inhibit the growth of normal commensals (unwanted bacteria).

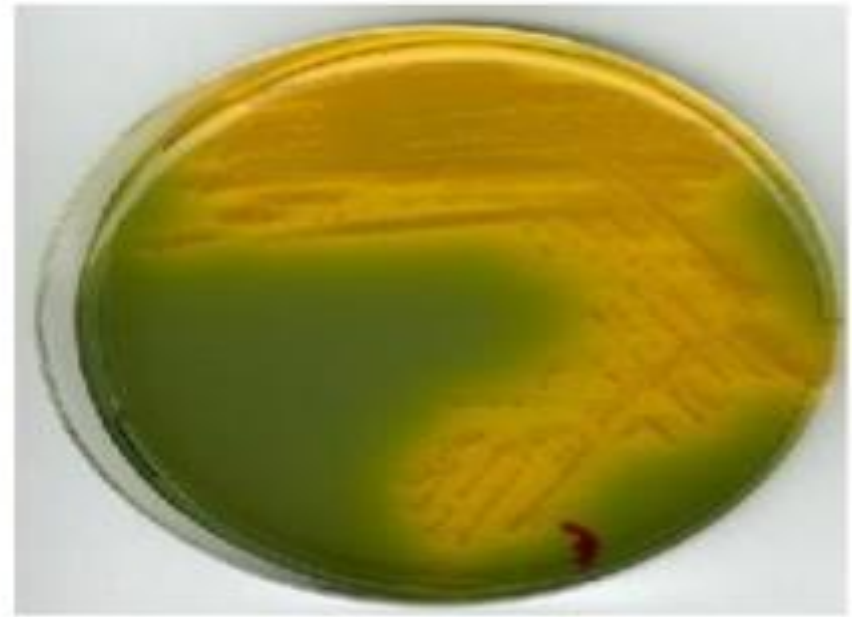
✓ *Vibrio chloerae* produce acid by fermentation of sucrose which acts on bromothymol blue (indicator) producing yellow colonies.



TCBS medium

(Thiosulphate Citrate bile salts sucrose agar)

For cultivation of Vibrio cholera



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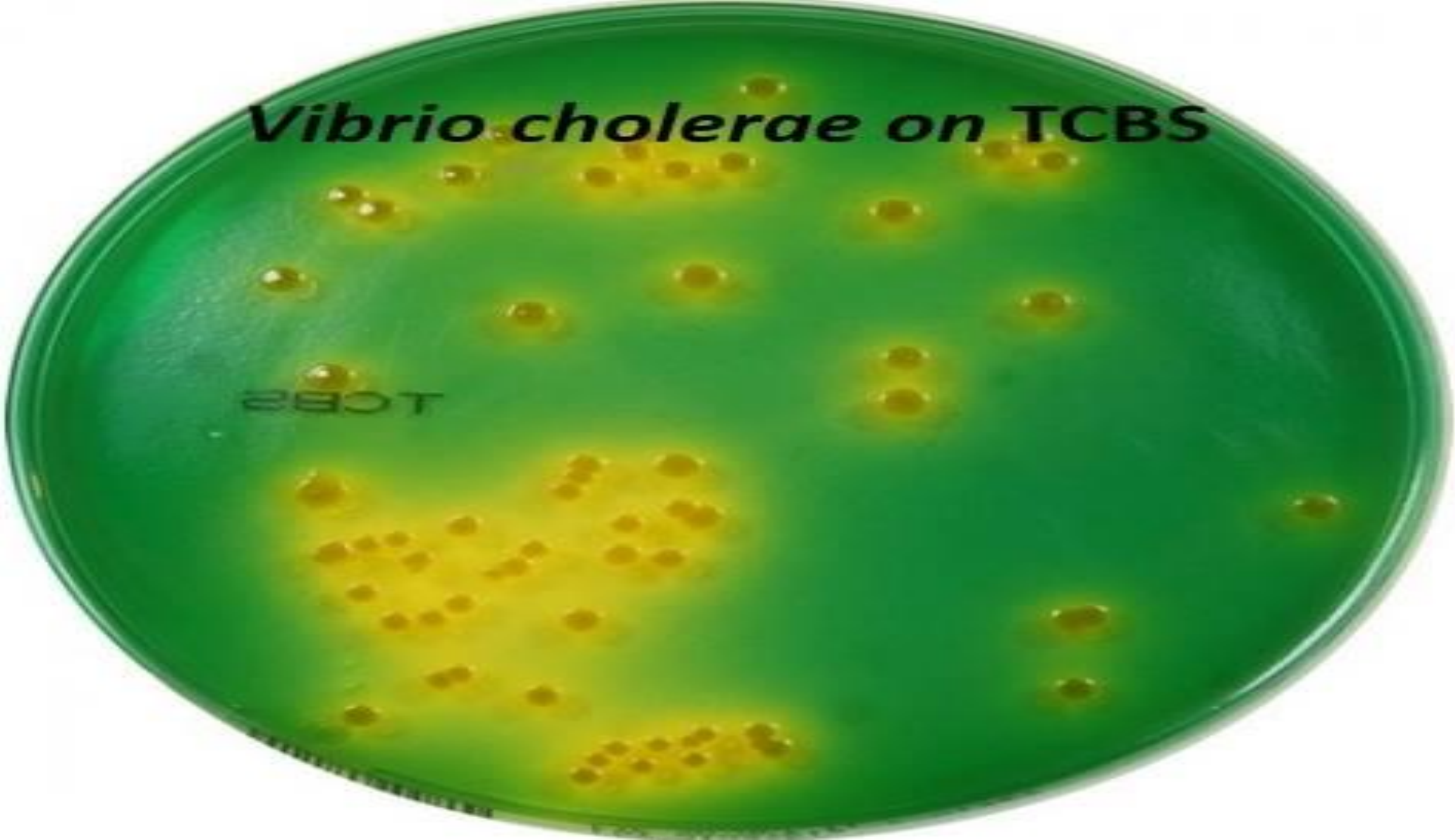
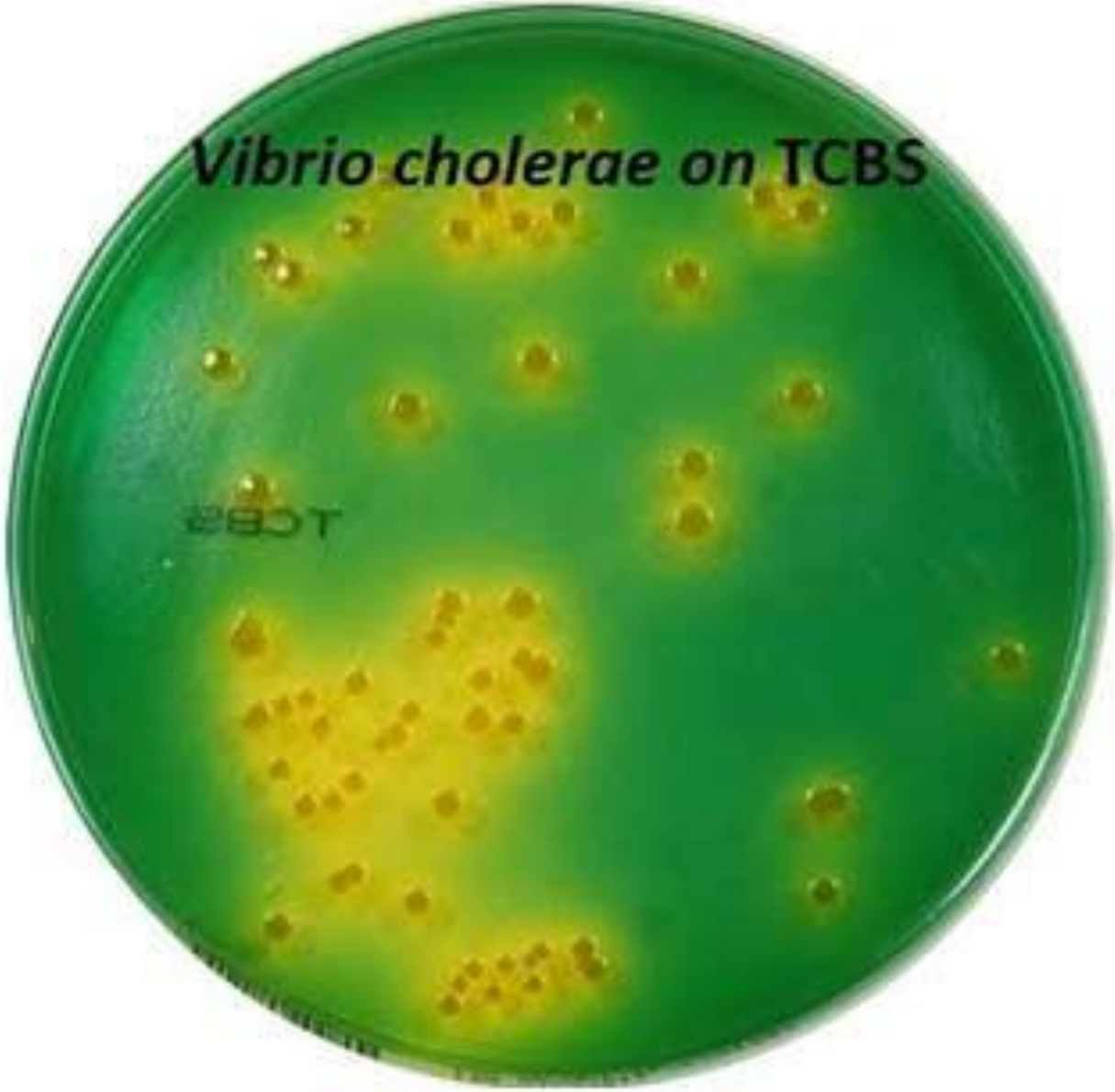

Vibrio cholerae on TCBS

V. Cholerae TCBS Agar

Appearance of Colonies	*Microorganisms*
Flat, yellow	*Vibrio cholerae*
Small, blue-green centre	*Vibrio parahaemolyticus*



Vibrio cholerae on TCBS Agar

Vibrio parahaemolyticus on TCBS Agar

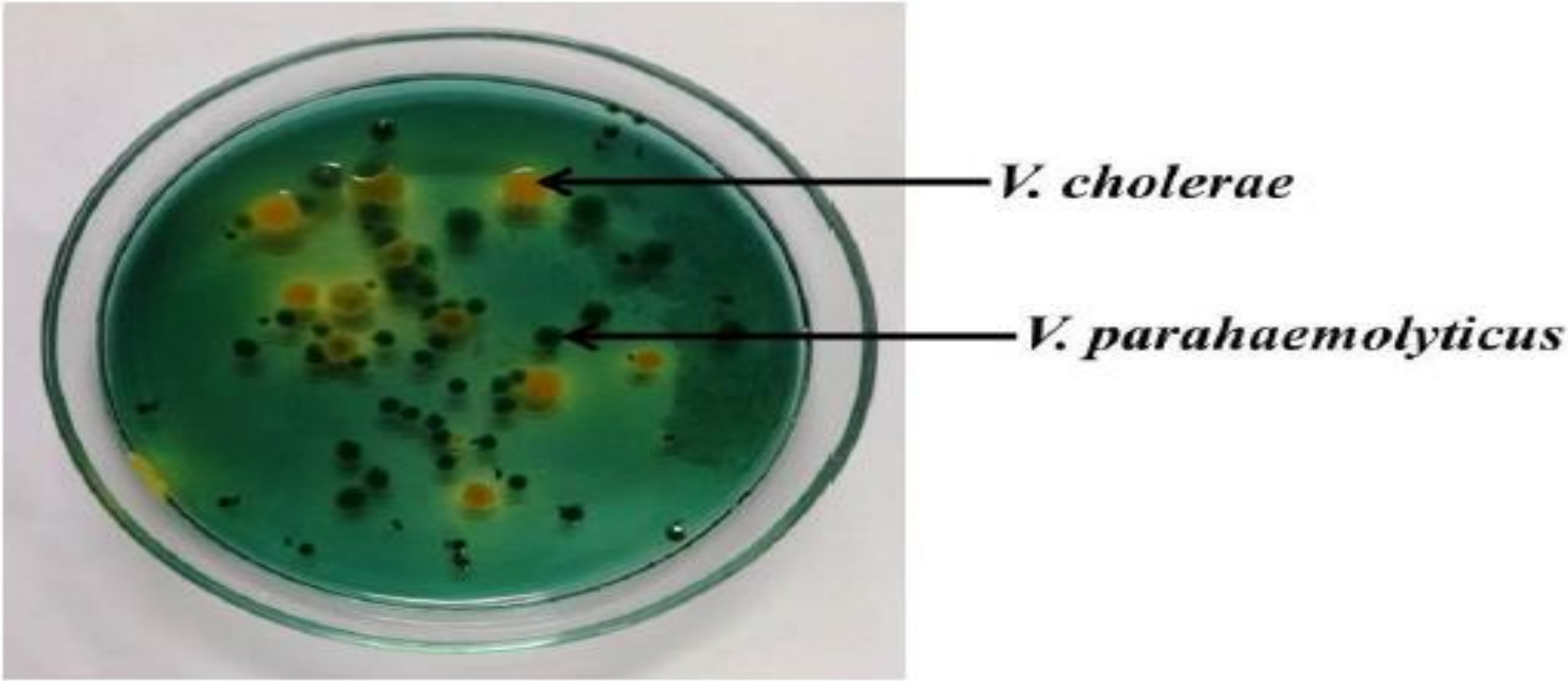


Figure 3. Bacterial colonies grown on TCBS agar medium isolated from Pariyat reservoir, Jabalpur. Large yellow colonies represent growth of *V. cholerae* and small green colonies represent *V. parahaemolyticus*.

Test	Vibrio cholera	Vibrio parahaemolyticus	
Catalase and oxidase	+	+	
NO2 reduction	+	+	
Indol	+	+	
MR	+weak		
VP	-	-	
SC	V	V	
Peptone water 7% NaCl	-	+	
Peptone water 0% NaCl	+	-	
TSI	A\A ,_,_	K\A,_,_	
Motility	+	+	
Cholera red	+	-	
Mannitol	+weak	+weak	
String test	+	+	
OF media	Oxidation and fermentation	Oxidation	

Specific tests for Vibrio cholerae

- String test – (+)
- Cholera Red Reaction – (+)
- Gelatin liquefaction – (+)

Other reactions of Vibrio cholerae

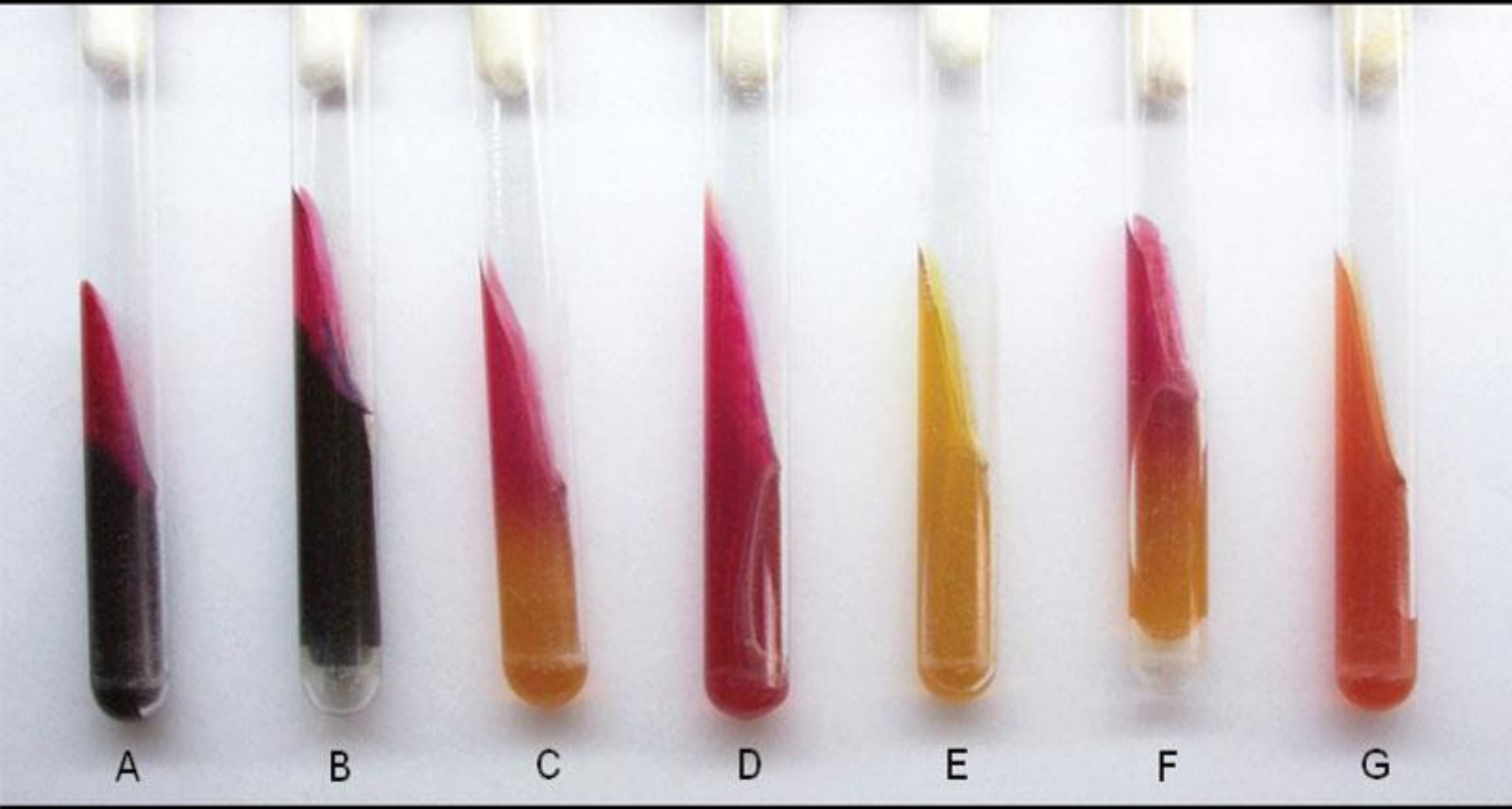
MR – (-)

VP – (+/-)

Citrate (+)

Urease – (-)

TSI – A/A, Gas (-), H_2S (-)


A
B
C
D
E
F
G